A phylogenetic analysis of the Smilacaceae based on morphological data

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Abstract   In order to construct phylogenetic relationships of the family Smilacaceae, 79 species representing all genera and sections of the family from all over the world were used for cladistic analysis, with *Philesia* and *Lapageria* of Philasiaceae as outgroups. A matrix of 36 binary and 11 multistate morphological characters was constructed. The first parsimonious analysis yielded numerous most parsimonious trees of 275 steps, and then 82 most parsimonious trees with 40.729 steps length were produced by reweighting characters using maximum values of rescaled consistency (RC), which remained stable after the second reweighting. There is a moderate resolution but generally poor bootstrap support for the phylogenetic relationships in the family. From the strict MP tree and the NJ tree, *Ripogonum* is a sister to *Smilax*, but may be better treated as a separate family. *Heterosmilax* is considered to be derived from ancestral *Smilax* and occurs as the most derived clade of the Smilacaceae, and thus it is reasonable to retain it as a section in *Smilax* s.l. Within *Smilax* most currently recognized sections are polyphyletic, but some major lineages are distinct. The herbaceous *Smilax* species form a monophyletic clade, and it is also the case for the South American species. Their positions in the cladogram are intriguing and may help to shed light on evolutionary and biogeographic history of the family.
Key words   Smilacaceae, *Smilax*, *Heterosmilax*, *Ripogonum,* morphology, cladistics, phylogeny.

The family Smilacaceae was separated by Hutchinson (1973) from the polyphyletic family Liliaceae s.l., and this treatment was followed by a number of botanists (Dahlgren, 1975; Dahlgren et al., 1985; Cronquist, 1981, 1988; Thorne, 1983; Takhtajan, 1987; Conran, 1998; Judd, 1998; Judd et al., 1999). It comprises ca. 375 species (Heywood et al., 1993) with a wide distribution in tropics and subtropics. The family is characterized by reticulate leaf venation, paired petiolar tendrils, dioecious flowers, umbellate inflorescences and mostly woody, climbing habit. Although there is a general agreement on the familial status of the Smilacaceae, the controversy on its circumscription and infrafamilial phylogenetic relationships still exists among botanists. Dahlgren (1975) included four genera in the family, viz., *Smilax* L.*, Heterosmilax* Kunth.*, Pseudosmilax* Hayata and *Ripogonum* J. R. Forst. & G. Forst., whereas Takhtajan (1997) recognized only two genera, *Smilax* (including *Pseudosmilax*) and *Heterosmilax*. Conran & Clifford (1985) suggested for the first time that *Ripogonum* be placed in a family of its own based on the phenetic studies, but this suggestion has not been widely accepted. Recent molecular data (Chase et al., 2000; Cameron & Fu, in press) indicated that *Ripogonum* was a sister to *Smilax*. However, Rudall and her coworkers

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(Rudall et al., 2000) constructed a combined tree from molecular and morphological data, showing that *Smilax* is a sister group to the *Philesia-Ripogonum* complex. Therefore, the circumscription and generic relationships of the family remain to be clarified. The Smilacaceae is here circumscribed as containing three genera, *Smilax, Heterosmilax*, and *Ripogonum* following Koyama (1960, 1984), Hutchinson (1973), Heywood et al. (1993), Mabberley (1997), Takhtajan (1997), and Conran (1998).

    Smilax is a core genus of the family with ca. 350 species (Takhtajan, 1997) or ca. 200 species (Cameron & Fu, in press) and worldwide distribution, but most diverse in Asia and America. *Heterosmilax,* with ca. 12 species, is a small southeastern Asian genus closely allied to *Smilax*, while *Ripogonum* with six species occurs only in eastern Australia, New Guinea, and New Zealand. In his attempt at monographic study of the family, Koyama (1960) proposed a classification of six sections in *Smilax*, and later he (Koyama, 1984) divided *Heterosmilax* into two sections. Holmes (2002) suggested without explanation that *Heterosmilax* be reduced to a section within *Smilax.* Conran and Clifford (1985) considered *Ripogonum* distantly related to the rest of the Smilacaceae based on phenetic analyses. The taxonomic treatments of the family and genera in the past several decades (Wang et al., 1978; Andreata, 1997; Judd, 1998; Chen et al., 2000) involved mainly local areas, and thus the family has never been monographed in its entirety and is in need of monographic and phylogenetic study.

    As a part of phylogenetic research of the Smilacaceae, this paper attempts to construct a phylogeny of the family through cladistic analysis based on morphological data. The paper describes variability of morphological characters in 79 species with two genera (*Philesia* Comm. ex Juss. and *Lapageria* Ruiz & Pav.) of the family Philesiaceae as outgroups.

1   Material and methods

1.1   In-group and outgroups

    Seventy-nine species of the three genera were selected as operational taxonomic units (OTUs) or terminal taxa. The present analysis was based on the study of herbarium specimens from the Herbaria of Institute of Botany, the Chinese Academy of Sciences (PE); Kunming Institute of Botany, the Chinese Academy of Sciences (KUN); Zhejiang University, China (HZU); New York Botanical Garden, the United States (NY); Muséum National d'Histoire Naturelle, Paris, France (PCU); and University of Tokyo, Japan (TI). Living material in the field in China and the United States and transplanted materials growing in the Botanical Garden of Zhejiang University were also used. The information of partial characters was taken from the literature. The voucher specimens are listed in Table 1.

    Two outgroups were chosen based on the system of APGII (2003). In addition, Philesiaceae is positioned as sister to Smilacaceae following the cladistic analyses on monocots using morphological and *rbc*L sequence data performed by Chase et al. (1995). Thus, two species respectively from the genera *Philesia* and *Lapageria* of Philesiaceae were chosen as the outgroups. All the trees in this paper were rooted with this family.

1.2   Morphological characters

    In order to minimize the noise due to developmental plasticity, every effort was made by using mature organs for each character. Forty-seven vegetative and floral characters, including some palynological and cytological characters, were selected in this analysis, of which 36 were scored as binary and 11 as multi-state (Table 2). In a few cases, some characters were scored as missing when unavailable. Winclada program (Nixon, 1999) was employed to record raw data and create data matrix for tree-building calculation. The complete data matrix is shown in Table 3.

Table 1   Source of materials for observation

Taxon	Locality	Voucher
Heterosmilax chinensis Wang	Without precise locality, Sichuan ( ), China	W. C. Cheng ( ) 11002 (PE)
H. gaudichaudiana (Kunth) Maxim.	Without precise locality, Guangdong ( ), China	S. Y. Hu ( ) 12870 (PE)
H. japonica Kunth	Taishun ( ), Zhejiang ( ), China	C. X. Fu ( ) 96118 (HZU)
H. seisuiensis (Hayata) Wang & Tang	Without precise locality, Taiwan ( ), China	Anonymous s.n. 82439 (PE)
H. yunnanensis Gagnep.	Xichou ( ), Yunnan ( ), China	S. Z. Wang ( ) 0192936 (KUN)
Ripogonum album R. Br.	Without precise locality, New Guinea	Morobe & Distrikt 488 (NY)
R. discolor F. Muell.	Sydney, Australia	C. X. Fu ( ) 98020 (HZU)
Smilax aberrans Gagnep.	Without precise locality, Guizhou ( ), China	C. X. Fu ( ) 20037 (HZU)
S. arisanensis Hayata	Huaping ( ), Guangxi ( ), China	C. X. Fu ( ) 91052 (HZU)
S. aspera L.	Without precise locality, Italy	P. Delprete 7164 (NY)
S. aspericaulis Wall.	Gongshan ( ), Yunnan ( ), China	Dulongjiang Exped. ( ) 4707 (KUN)
S. astrosperma Wang & Tang	Mt. Wuzhishan ( ), Hainan ( ), China	X. R. Liang ( ) 64347 (PE)
S. auriculata Walt	Without precise locality, North Carolina, USA	B. Wrunctan 1197 (NY)
S. australis R. Br.	Without precise locality, Australia	L. Pv. 1196 (NY)
S. basilata Wang & Tang	Wenshan ( ), Yunnan ( ), China	S. C. Chen ( ) 0302029 (ZHU)
S. biumbellata Koyama	Without precise locality, Yunnan ( ), China	B. Y. Qiu ( ) 52520 (PE)
S. bona-nox L.	Without precise locality, North Carolina, USA	K. M. Cameron 1053 (NY)
S. bracteata Presl	Funing ( ), Yunnan ( ), China	H. T. Tsai ( ) 0193111 (KUN)
S. brasiliensis Spreng.	Without precise locality, Brazil	Anonymous s.n. 24826 (PCU)
S. californica A. Gray	Without precise locality, Virginia, USA	FW93 CF state 1913 (NY)
S. campestris Griseb.	Without precise locality, Brazil	From literature (Andreata, 1997)
S. chapaensis Gagnep.	Mt. Qingchengshan ( ), Sichuan ( ), China	C. X. Fu ( ) 88302 (HZU)
S. china L.	Pingbian ( ), Yunnan ( ), China	C. W. Wang ( ) 88478 (PE)
S. chingii Wang & Tang	Mt. Jinfoshan ( ), Nanchuan ( ), Chongqing ( ), China	C. X. Fu ( ) 92001 (HZU)
S. cissoides Mart. ex Griseb.	Without precise locality, Brazil	From literature (Andreata, 1997)
S. cocculoides Warb. ex Diels	Mt. Fanjingshan ( ), Guizhou ( ), China	C. X. Fu ( ) 91307 (HZU)
S. corbularia Kunth	Malipo ( ), Yunnan ( ), China	K. M. Feng ( ) 4871 (PE)
S. darrisii Lévl.	Nanchuan ( ), Chongqing ( ), China	K. Y. Lang ( ) 870 (PE)
S. davidiana A. DC.	Mt. Tianmushan ( ), Zhejiang ( ), China	S. C. Chen et al. ( ) 0306132 (HZU)
S. densibarbata Wang & Tang	Pingbian ( ), Yunnan ( ), China	C. W. Wang ( ) 85452 (PE)
S. discotis Warb.	Mt. Fengyangshan ( ), Zhejiang ( ), China	C. X. Fu ( ) 89144 (HZU)
S. ecirrhata Wats.	Without precise locality, USA	J. A. Steyermark 40460 (NY)
S. ferox Wall. ex Kunth	Without precise locality, Yunnan ( ), China	B. Y. Qiu & S. T. Li (,) 0193635 (KUN)
S. glabra Roxb.	Simao ( ), Yunnan ( ), China	Anonymous s.n. 6312, 0194040 (KUN)
S. glauca Walter	Without precise locality, New York, USA	C. X. Fu 9987 (HZU)
S. glauco-china Warb. ex Diels	Guilin ( ), Guangxi ( ), China	S. C. Chen ( ) 0304023 (HZU)
S. havanensis Jacq.	Without precise locality, Cuba	MMB770 (NY)
S. hayatae Koyama	Without precise locality, Taiwan ( ), China	Taipei Empire University ( ) 19308 (PE)
S. hemsleyana Craib	Wenshan ( ), Yunnan ( ), China	C. X. Fu ( ) 93043 (HZU)
S. herbacea L.	Without precise locality, North Carolina, USA	K. M. Cameron 11 (NY)
S. hispida Raf.	Gillies Lake, USA	P. V. Krotkov 7268 (NY)
S. jamesii G. A. Wallace	Without precise locality, California, USA	ARK 3725 (NY)
S. kraussiana Meisn.	Without precise locality, Gabon	JMR 3151 (NY)
S. lanceifolia Roxb.	Pingbian ( ), Yunnan ( ), China	C. W. Wang ( ) 71996 (PE)
S. lanceolata L.	Without precise locality, Mexico	E. Matuda 3988 (NY)
S. laurifolia L.	Without precise locality, Carolina, USA	P. Krotkov 5683 (NY)
S. lebrunii Lévl.	Xichou ( ), Yunnan ( ), China	S. C. Chen ( ) 03101417 (HZU)

Table 1 (continued)

Taxon	Locality	Voucher
S. lunglingensis Wang & Tang	Pingbian ( ), Yunnan ( ), China	B. Y. Qiu ( ) 52403 (PE)
S. lutescens Vell.	Without precise locality, Brazil	From literature (Andreata, 1997)
S. megacarpa A. DC.	Without precise locality, Cambodge, Thailand	V. N. Laos (PCU)
S. menispermoidea A. DC.	Pingbian ( ), Yunnan ( ), China	B. Y. Qiu ( ) 53633 (PE)
S. microphylla C. H. Wright	Mt. Jinfoshan ( ), Chongqing ( ), China	C. X. Fu ( ) 92027 (HZU)
S. minarum A. DC.	Without precise locality, Brazil	From literature (Andreata, 1997)
S. muscosa Toledo	Without precise locality, Brazil	From literature (Andreata, 1997)
S. myrtillus A. DC.	Gongshan ( ), Yunnan ( ), China	Dulongjiang Exped. ( ) 6680 (KUN)
S. nervo-marginata Hayata	Baishanzu ( ), Zhejiang ( ), China	S. C. Chen et al. ( ) 0308004 (HZU)
S. nigrescens Wang & Tang	Without precise locality, Sichuan ( ), China	S. C. Chen ( ) 02023 (PE)
S. nipponica Miq.	Kaili ( ), Guizhou ( ), China	Qiannan Exped. ( ) 1183 (KUN)
S. oblongifolia Pohl ex Griseb.	Without precise locality, Brazil	From literature (Andreata, 1997)
S. ocreata A. DC.	Pingbian ( ), Yunnan ( ), China	C. W. Wang ( ) 73366 (KUN)
S. outanscianensis Pamp.	Without precise locality, Hubei ( ), China	Y. Liu ( ) 530 (PE)
S. perfoliata Lour.	Yangshan ( ), Guangdong ( ), China	L. Deng ( ) 1543 (HZU)
S. planipes Wang & Tang	Pingbian ( ), Yunnan ( ), China	C. X. Fu ( ) 93015 (HZU)
S. polycephala Wang & Tang	Without precise locality, Yunnan ( ), China	Sino-USSR Exped. ( ) 2504 (PE)
S. polycolea Warb. ex Diels	Nanning ( ), Guangxi ( ), China	S. H. Chun ( ) 14559 (PE)
S. pottingeri Prain	Malipo ( ), Yunnan ( ), China	K. M. Feng ( ) 12162 (PE)
S. pumila Walter	Without precise locality, Florida, USA	K. M. Cameron 1065 (NY)
S. riparia A. DC.	Fengcheng ( ), Liaoning ( ), China	C. X. FU ( ) CF9902 (HZU)
S. rotundifolia Bello	Without precise locality, New York, USA	W. Stevens 23665 (NY)
S. scobinicaulis C. H. Wright	Mt. Emeishan ( ), Sichuan ( ), China	C. X. Fu ( ) 92051 (HZU)
S. sieboldii Miq.	Without precise locality, Zhejiang ( ), China	M. B. Deng ( ) 3914 (PE)
S. spicata Vell.	Without precise locality, Brazil	From literature (Andreata, 1997)
S. stans Maxim.	Zhenkang ( ), Yunnan ( ), China	T. T. Yu ( ) 11317 (PE)
S. syphilitica Willd	Without precise locality, Brazil	From literature (Andreata, 1997)
S. trinervula Miq.	Without precise locality, Jiangxi ( ), China	K. C. Kuan ( ) 77058 (PE)
S. tsinchengshanensis Wang	Nanchuan ( ), Chongqing ( ), China	K. H. Shing ( ) 937 (PE)
S. vanchingshanensis (Wang & Tang) Wang & Tang	Nanchuan ( ), Chongqing ( ), China	F. T. Wang ( ) 10914 (PE)
S. vitiensis A. DC.	Without precise locality, Fiji	T. G. Yuncker 15512 (NY)
S. walteri Pursh	Without precise locality, North Carolina, USA	KMC1058 (NY)
Lapageria rosea Ruiz & Pav.	Without precise locality, Chile	MFD3686-6 (NY)
Philesia buxifolia Lam. ex Poir.	Without precise locality, Chile	KMC01 (NY)

1.3   Phylogenetic analysis

Maximum parsimony (MP) and neighbor-joining (NJ) trees were constructed using PAUP* 4.0b10 program (Swofford, 2002). Cladistic analysis of phylogenetic relationships was conducted by using Wagner parsimony and applying heuristic search with tree bisection-reconnection (TBS) branch-swapping, multiple parsimony (MUL-PARS), and simple stepwise taxon application of 1000 replications. During the first tree-construction process, all characters were unordered and equally weighted with multi-states interpreted as uncertainty, and "?" treated as missing information. Subsequently, reweighting of the characters by maximum values of rescaled consistency indexes (*RC*) was performed in order to reduce the misleading effect of homoplasious characters. Phenetic analysis was also performed with the PAUP* program by using the neighbor-joining clustering method, with a mean character difference as a distance measure. Supporting values were calculated by performing bootstrap (bts) analysis of 1000 heuristic search replicates with the TBR branching swapping algorithm (Felsenstein, 1985).

Table 2   List of characters and character states used in data matrix

1. Habit : 0—woody, 1—herbaceous	24. Receptacle: 0—obviously bulgy, 1—not bulgy
2. Stems aboveground: 0—perennial, 1—annual	25. Number of flowers per umbel: 0— 10, 1—>10
3. Stem: 0— vines, 1—erect	26. Filament length: 0— 2.5 mm, 1—<2.5 mm
4. Cross section of stem 0—round, 1—square	27. Cross section of peduncle: 0—round, 1—compressed
5. Wing on stem: 0—absent, 1—present	28. Rachis length: 0—obviously shorter than petiole, 1—subequal to petiole, 2—longer (obviously longer than petiole)
6. Prickles on stem: 0—absent, 1—present	
7. Shape of prickles on stem: 0—blackish needle-like, 1—triangular or obtuse or absent	29. Bract on peduncle: 0—absent, 1—present
8. Verrucae on stem: 0—absent, 1—present	30. Stamen length/perianth length: 0—subequal, 1—1/2
9. Tendril: 0—absent, 1—present	31. Number of stamens in male flowers: 0—three, 1—six, 2—more than six
10. Leaf margin: 0—regular, 1—irregular (minutely roughened under a lens)	
	32. Number of staminodes in female flower: 0—six, 1—three, 2—absent
11. Leaf color when dry: 0—yellowish or greenish, 1—blackish	
	33. Mature stamens: 0—spreading into a plane, 1—otherwise
12. Phyllotaxis: 0—alternate, 1—opposite or whorled	34. Filament status: 0—free, 1—partly connate, 2—connate
13. Abscission zone of leaf on petiole: 0—at middle, 1—at top, 2—at bottom	35. Tepals: 0—free, 1—connate
14. Leaf blade white hairy abaxially: 0—absent, 1—present	36. Tepals when mature in male flower: 0—recurved, 1—erect
15. Number of main veins: 0—seven, 1—five, 2—three	37. Flowering time: 0—March–July, 1—August–December
16. Wings on petiole: 0—absent, 1—narrow, 2—semicircular, 3—broadly clasping node	38. Shape of berries: 0—globose, 1—prolate
	39. Carpopodium: 0—straight, 1—recurved
17. Wing length/petiole length: 0— 1/2, 1—>1/2	40. Berry diameter: 0—small (1 cm), 1—big (>1 cm)
18. Flowers: 0—bisexual, 1—monosexual	41. Berry color: 0—black, 1—red
19. Flowers borne on stem: 0—solitary, 1—as an inflorescence	42. Tepal color: 0—greenish-yellow, 1—red-brown
20. Inflorescence: 0—single umbels, 1—panicle, 2—spike	43. Pollen aperture: 0—monosulcate, 1—inaperturate, 2—pseudo-aperturate
21. Rachis articulation: 0—absent, 1—present	44. Pollen ornamentation: 0—reticulate, 1—spinulate, 2—rugulate
22. Rachis arising: 0—at young branch, 1—in axil of leaf	45. Density of spinules on pollen: 0—low (<90 per 100 μm^2), 1—high (>90 per 100 μm^2)
23. Scale-like prophyll at base of peduncle: 0—absent, 1— present	46. Length of spinules on pollen: 0—long (>3 μm), 1—short (<3 μm)
	47. Basic numbers of chromosomes (n=): 0—16, 1—13, 15

2   Results

Cladistic analysis of the matrix with 47 morphological characters gave more than 154000 equally most parsimonious trees of 275 steps length with RC=0.141 (consistency index CI=0.218, retention index RI=0.647). Only six clades were found in the strict consensus tree (not shown). The low RC value indicates a high level of homoplasy in the variation pattern of morphological characters. Farris (1969, 1989) suggested that homoplasious characters be downweighted to obtain reliable result and proposed successive reweighting of characters by RC to reduce the ambiguity caused by homoplasious characters. Twice reweighting characters by maximum values of RC yielded 82 most parsimonious trees with 40.729 steps length (CI=0.513, RI=0.840, RC=0.431) which remained stable after the second reweighting. A strict consensus of 82 reweighted equally parsimonious trees is shown in Fig. 1. The unweighted and reweighted trees revealed essentially the same clades of species clusters except position of a few species.

Table 3   Data matrix employed in the cladistic and phenetic analysis of Smilacaceae

	5	10	15	20	25	30	35	40	45
Heterosmilax chinensis	00000	00110	01011	10110	00001	11100	01111	00010	21121	10
H. gaudichaudiana	00000	00010	01011	10110	00001	01200	0–121	0?010	00121	10
H. japonica	00000	00010	01110	10110	00001	01110	01111	01010	00121	10
H. seisuiensis	00000	00010	01111	10110	00001	11000	2–101	00010	00121	10
H. yunnanensis	00000	00010	01100	10110	00001	01100	20111	00010	00121	10
Ripogonum album	00000	00000	00011	00012	00011	00200	1–100	10011	10000	1?
R. discolor	00000	10000	00011	00012	00011	00200	1–100	10011	10000	1?
Smilax aberrans	00100	00000	01112	11110	01011	10201	10100	10001	00110	00
S. arisanensis	00000	10010	01111	11110	00111	10200	11100	00010	00110	00
S. aspera	00000	00010	01100	10112	00101	10201	10100	01010	10110	00
S. aspericaulis	00000	10110	01011	10111	00101	00110	11100	11110	01110	00
S. astrosperma	00000	10010	01012	10110	00101	00011	11100	1?010	00110	0?
S. auriculata	00000	10010	01011	10110	00001	01000	10100	00010	00110	0?
S. australis	00000	10010	01012	10110	10101	11100	1?120	0?010	00110	0?
S. basilata	00000	10010	01112	11110	00100	10000	1?120	00011	00110	0?
S. biumbellata	00000	00010	01110	10110	00000	10200	11100	00010	01110	00
S. bona-nox	00000	11110	01000	10110	00001	01200	1?100	10010	10110	0?
S. bracteata	00000	10010	01011	10111	00101	00110	11100	11010	?1110	00
S. brasiliensis	00000	10010	01001	10110	01001	10001	10000	2?010	?0210	0?
S. californica	00000	11011	01010	10110	00001	01200	10100	1?010	00110	0?
S. campestris	00000	00010	01212	10110	00001	10201	12000	2?010	?0210	0?
S. chapaensis	00000	10110	01011	10110	10101	00010	10100	11010	00110	00
S. china	00000	10010	01011	11110	01001	00200	10100	10010	10110	00
S. chingii	00000	10010	01011	11110	01010	00200	10100	10010	10110	00
S. cissoides	00000	10010	01111	10110	00001	10101	10000	2?010	?0210	0?
S. cocculoides	00100	00000	01012	10110	10100	10110	1–120	00010	00110	00
S. corbularia	00000	00010	01111	10110	00001	11101	11121	01010	10121	00
S. darrisii	00100	00000	11112	10110	00010	10201	1?100	00010	00110	0?
S. davidiana	00000	10010	01111	21110	01001	00200	11100	10010	10110	00
S. densibarbata	00000	10010	01111	10110	10101	00111	10100	00010	00110	0?
S. discotis	00000	10010	01111	21110	01000	00200	11100	00010	00110	00
S. ecirrhata	11100	00010	01110	10110	00001	01200	10100	10010	00110	01
S. ferox	00000	10000	01112	21110	01011	00100	10100	10010	10110	00
S. glabra	00000	00010	01112	10110	00001	10001	11110	01010	00121	00
S. glauca	00000	11010	01111	11110	00010	11200	10100	00010	00110	0?
S. glauco-china	00000	10010	01111	11110	01001	00200	11100	00010	00110	00
S. havanensis	00000	10010	01101	10110	01001	11001	10100	1?010	00110	0?
S. hayatae	00000	00000	01112	10110	00010	10201	1?100	00010	10110	0?
S. hemsleyana	00000	00010	01010	10110	10101	00110	11110	10010	?0110	0?
S. herbacea	11000	00010	01110	10110	00001	01200	10100	10010	00110	01
S. hispida	00000	11011	01110	10110	00001	00200	1?100	10010	00110	00
S. jamesii	11100	00010	01211	10110	00001	01200	10100	10010	00110	01
S. kraussiana	00000	10010	01111	10110	10101	01110	1?100	0?010	?0110	0?
S. lanceifolia	00000	10010	01011	10110	10101	00010	10100	11010	00110	00
S. lanceolata	00000	10010	01111	10110	00001	01100	11100	11010	00110	0?
S. laurifolia	00000	10011	01011	10110	00001	00100	10100	01010	00110	0?
S. lebrunii	00000	10110	01111	11110	01000	00100	10100	10010	10110	00
S. lunglingensis	00000	10010	01011	11111	00101	00110	11100	10010	00110	00
S. lutescens	00000	00010	01012	10110	00001	10100	1?000	2?010	??210	0?
S. megacarpa	00000	00010	11111	10110	10100	00110	11100	11011	10110	0?
S. menispermoidea	00000	00010	01111	11110	00000	10201	10120	00010	01110	00
S. microphylla	00000	10010	01111	11110	00001	10001	11100	01010	00121	00
S. minarum	00000	00110	01011	10110	00011	10201	12000	2?010	??210	0?
S. muscosa	00000	10111	01111	10110	01001	10000	1?000	2?010	??210	0?
S. myrtillus	00101	10000	01012	11110	00010	10201	1?100	01010	00110	00
S. nervo-marginata	00000	00110	01110	10110	00000	11201	1?100	00010	00121	00
S. nigrescens	00000	10010	11111	11110	00001	10201	10100	00010	00110	0?
S. nipponica	11000	00010	01110	10110	00001	01200	10100	10010	00110	01
S. oblongifolia	00100	00000	01111	10110	00001	10100	11000	2?010	?0210	0?
S. ocreata	00000	10010	01011	31111	00101	00111	12110	10010	10110	00
S. outanscianensis	00000	10010	01011	10110	01010	00100	10100	10010	00110	0?
S. perfoliata	00000	10010	01011	31111	00101	00111	12100	10010	10110	0?
S. planipes	00000	00010	01111	11110	10110	00110	11100	11011	?0110	0?
S. polycephala	00011	00010	01111	10112	00101	10211	1–100	01011	00110	00
S. polycolea	00000	10010	01011	10110	01000	00200	10100	00010	10110	00

Table 3   (continued)

	5	10	15	20	25	30	35	40	45	
S. pottingeri	00000	00010	11011	10110	00001	11200	1–100	00010	00110	0?
S. pumila	00000	00110	01211	10110	00001	11000	10100	11110	10110	0?
S. riparia	11000	00010	01110	10110	01001	01200	10100	10010	00110	01
S. rotundifolia	00000	10010	01010	10110	00000	00100	11100	10011	00110	0?
S. scobinicaulis	00000	11010	11110	11110	00010	00000	11100	10010	00110	00
S. sieboldii	00000	11011	01110	10110	00010	00200	11100	10010	00110	00
S. spicata	00011	00010	01111	10110	01001	10100	10100	2?010	?0210	0?
S. stans	00100	00000	01111	11110	00010	10201	10100	00010	00110	00
S. syphilitica	00000	10110	01012	30110	10101	10210	1?000	2?010	?0210	0?
S. trinervula	00000	10010	01012	10110	01010	00100	10100	10010	10110	00
S. tsinchengshanensis	00100	00000	01112	11110	01110	10201	11100	01010	01110	00
S. vanchingshanensis	00000	00010	01011	10110	10111	00110	10110	11010	?0110	00
S. vitiensis	00000	00010	01211	10110	00001	00100	2?100	1?01?	?0110	0?
S. walteri	00000	10010	01011	10110	00010	00100	10100	10110	10110	0?
Lapageria rosea	00000	00000	01111	0000–	–00—	00100	1–100	0?011	?11??	?1
Philesia buxifolia	00000	00000	01112	0000–	–00—	00100	1–100	0?011	?11??	?1

Phenetic neighbour-joining analysis on the same matrix yielded a tree of 257 steps length and with *CI*=0.213 and *RC*=0.676 (Fig. 2). The neighbour-joining and parsimonious trees revealed similar clades of species, which differed in the position of some *Smilax* species.

In the strict consensus tree (Fig. 1) and the NJ tree (Fig. 2), the *Ripogonum* was basal to the whole cladogram, in which *R. album* and *R. discolor* formed a monophyletic clade with 100% and 99% bootstrap values respectively. The two species are characterized by opposite or whorled leaves (character 12), hermaphrodite flowers (character 18), and monosulcate and reticulate pollen (character 43, 44).

All species of *Smilax* and *Heterosmilax* appeared in a clade on both the strict consensus tree (Fig. 1) and the NJ tree (Fig. 2), with a high bootstrap value (>80%). This clade was well circumscribed and characterized by a number of characters, especially two synapomorphies, i.e., a pair of tendrils (character 9) and dioecism (character 18). On the strict tree, the clade of five *Heterosmilax* species was supported by a high bootstrap value (84%), and merged in a bigger clade including *S. corbularia*, *S. nervo-marginata*, *S. glabra* and *S. microphylla* with 89% bootstrap value, but the value of the bigger clade reduced to 37% in the NJ tree.

The herbaceous species, disjunct in East Asia and North America, always formed a strongly supported clade on both the strict tree and the NJ tree (bootstrap values 93% and 86% respectively), with two synapomorphies, herbaceous habit (character 1) and annual stem (character 2).

In both the strict tree and the NJ tree, the woody species from South America joined in a clade. They share characters of petioles with narrow wings (character 16) and tepals of mature male flowers spreading into a plane (character 33). This clade was supported by 69% bootstrap value in the strict tree, but 23% in the NJ tree.

In the present analysis, many characters had a high level of homoplasy with low consistency indices. Some portions of the trees were unresolved with low bootstrap values, and much resolution was lost within the strict tree.

3   Discussion

In recent years, most botanists adopted a narrow concept of the Smilacaceae with only three genera, *Smilax*, *Heterosmilax*, and *Ripogonum* (Thorne, 1992; Heywood et al., 1993; Takhtajan, 1997), but Cronquist (1981, 1988) accepted a broader definition, including *Smilax*, *Ripogonum* and Philesiaceae, Luzuriagaceae, and Petermanniaceae. However, as indicated in the recent phylogenetic analysis of molecular data by Chase and his coworkers (Chase et al., 1993, 1995, 2000), the family as circumscribed by Cronquist (1988) is very probably

polyphyletic. In this study we follow the narrow concept of the family.
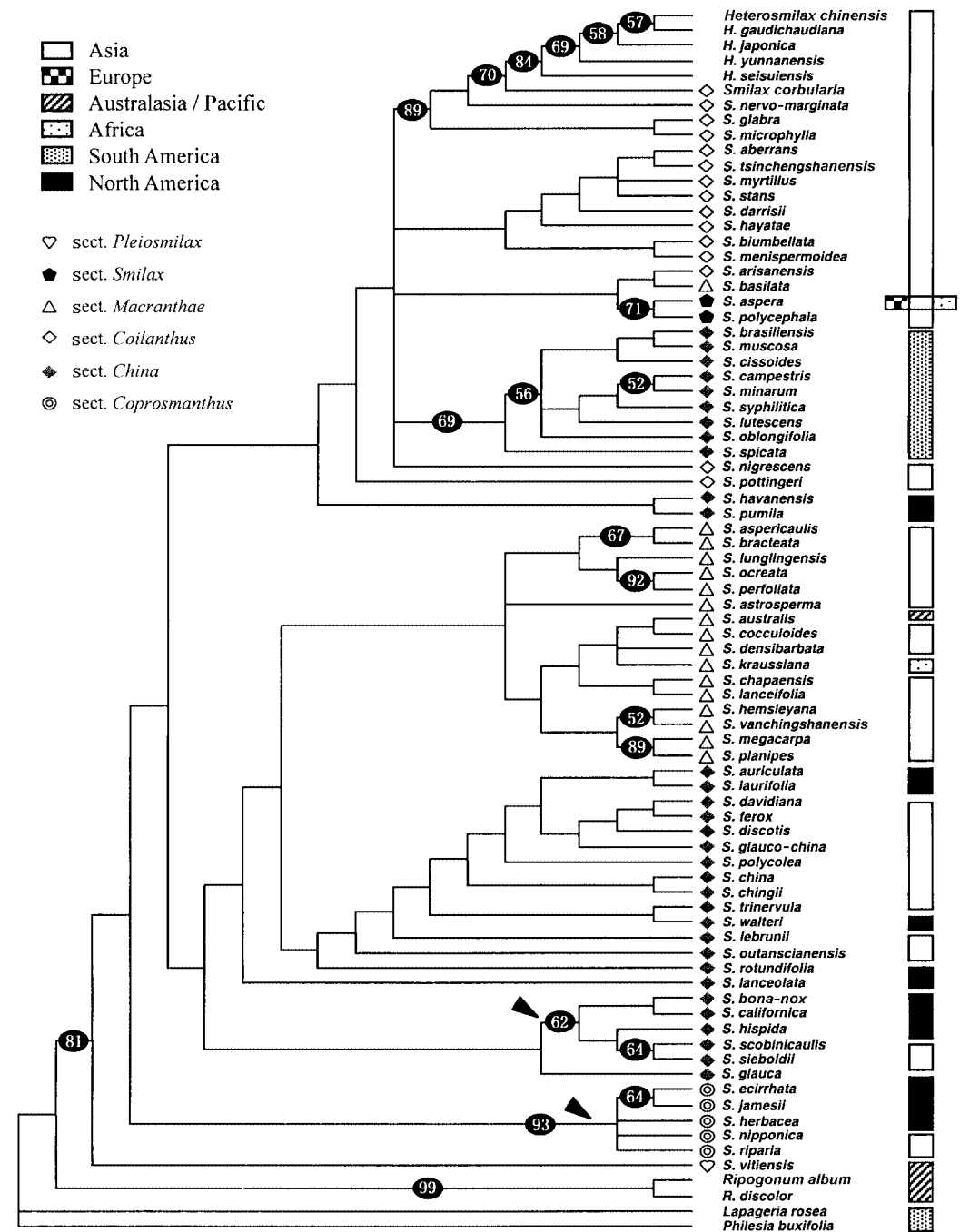

Fig. 1. The strict consensus tree of 82 equally most-parsimonious cladograms obtained from phylogenetic analysis of morphological data for Smilacaceae. The tree is oriented with *Lapageria rosea* and *Philesia buxifolia* (Philesiaceae) as the designated outgroups. Numbers on branches are bootstrap values (Bootstrap values below 50% are not shown) and geographic distribution and the previous section for each species are indicated.
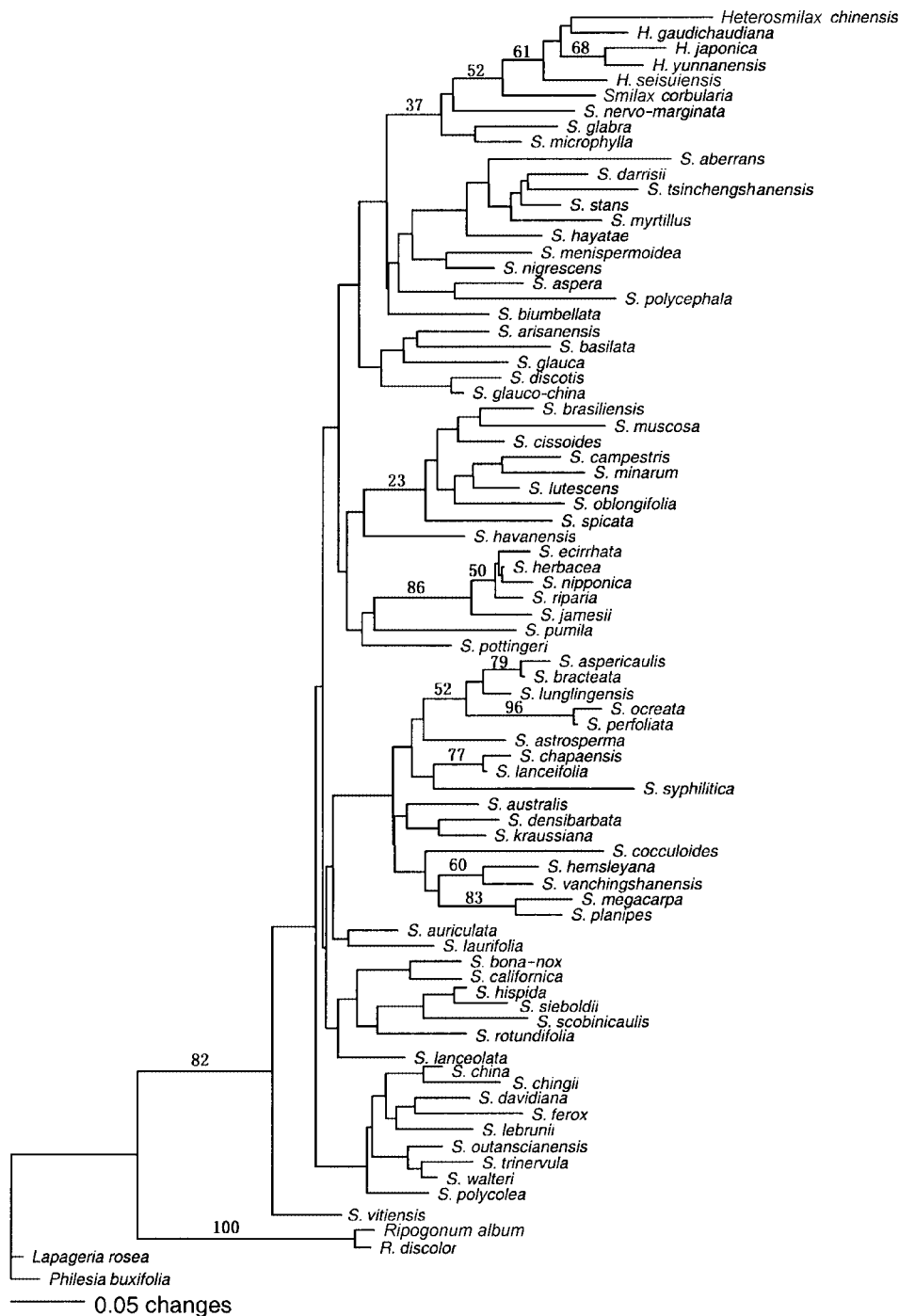
Fig. 2. The neighbor-joining tree obtained from phylogenetic analysis of morphological data for Smilacaceae. Numbers above branches are bootstrap values (Bootstrap values below 50% are not shown).

3.1   Phylogenetic position *Ripogonum*

The placement of *Ripogonum* in the Smilacaceae has been questioned by some botanists (Conran & Clifford, 1985; Conran, 1987, 1989; Takhtajan, 1997). Based on 56 morphological characters, Conran & Clifford (1985) found that *Ripogonum* is in phenetics closer to the Dioscoreaceae than to *Smilax* and *Heterosmilax*. The recent ITS analysis of *Smilax* (extending to the whole family) by Cameron & Fu (in press) shows that *Ripogonum* is a sister to the remaining two genera *Smilax* and *Heterosmilax*. The present result shows that *Ripogonum* species form a clade with a high bootstrap value (99%) in the strict tree (Fig. 1), and is also sister to *Heterosmilax* and *Smilax*. Furthermore, in the NJ tree (Fig. 2), the branch separating *Ripogonum* from *Smilax* is more than five times longer than the branch between *S. vitiensis* and the remaining species of *Smilax*, which implies a somewhat isolated position of *Ripogonum* within the family. The genus with six species is found only in Eastern Australia, New Guinea and New Zealand and is characterized by monosulcate and reticulate pollen, bisexual flowers, opposite/whorled leaves, and absence of paired tendrils. The separation of *Ripogonum* as an independent family by Conran & Clifford (1985), followed by Takhtajan (1987), is also supported by our study.

The APGII authors (APGII, 2003) mentioned that the Ripogonaceae and Philesiaceae may be included in the Smilacaceae on account of their spinose pollen. Actually, pollen of *Ripogonum* is monosulcate and reticulate rather than inaperturate and spinose according to our palynological investigation of the Smilacaceae (Chen et al., unpublished). Fay (in press) proposed that the three families would be best treated separately since they do not form a monophyletic group within the combined molecular analyses.

3.2   Phylogenetic position *Heterosmilax*

Heterosmilax was established by Kunth (1850) and accepted in a monographic treatment of de Candolle (1878), based on possession of a fused perianth. Its taxonomy was subsequently revised by Koyama (1984). However, the generic status of *Heterosmilax* has recently been questioned by some workers (Judd, 1998; Holmes, 2002). In the strict tree (Fig. 1), although all the species of *Heterosmilax* form a clade, they are nested in *Smilax* and form a larger clade with four species of *Smilax*, which indicates that they are not in a position to deserve a separate genus. A few species of *Smilax* (e.g. *S. corbularia*) have a partially united perianth (Chen et al., 2000) and gradation in pollen morphology exists between *Heterosmilax* and *Smilax* (Chen et al., unpublished). These two facts make the establishment of *Heterosmilax* as a genus unjustifiable. Judd (1998), Judd et al. (1999), Takhtajan (1997) and Holmes (2002) maintained the group in *Smilax*, which was confirmed by ITS analysis (Cameron & Fu, in press) and this study.

In the strict tree, all the species of *Heterosmilax* form a most derived clade, which implicates that evolutionary direction is from *Smilax* to *Heterosmilax*.

3.3   Phylogenetic relationships within *Smilax*

Smilax is a core genus in the family with ca. 200 species, including both woody and herbaceous species. Koyama (1960) recognized six sections in his taxonomic treatment of eastern Asian *Smilax*: sect. *Pleiosmilax*, sect. *Smilax*, sect. *Macranthae*, sect. *Coilanthus*, sect. *China*, and sect. *Coprosmanthus* (*Nemexia*). However, a recent study on the genus using ITS sequences (Cameron & Fu, in press) shows that Koyama's system is possibly artificial. The strict tree presented here also shows that Koyama's sections, except for sect. *Coprosmanthus* (*Nemexia*), are not supported as monophyletic groups (Fig. 1). The section *Coprosmanthus* (*Nemexia*) is characterized by herbaceous habit and annual stem, and is a sister to all woody *Smilax* (except *S. vitiensis*). This result is consistent with the results of ITS and *rpL*16 analyses (Fu et al., 2005).

The two species in sect. *Smilax*, *S. aspera* and *S. polycephala*, also form a small clade.

However, this result conflicts with the ITS analysis, which indicates that *S. aspera*, the most widely distributed species in the genus, is a sister to the entire family, and with the most primitive position (Cameron & Fu, in press). The exact position of *S. aspera* in the family needs further investigation. In sect. *Pleiosmilax*, only one species from Fiji, *S. vitiensis*, was analyzed in the present study, and thus it cannot be judged as monophyletic or not. In the strict tree, this Pacific species possessing numerous stamens is at the basal position of *Smilax*, which is unexpected. At any rate, we still presume that the feature of numerous stamens has evolved at least twice in the Smilacaceae, since three species of *Heterosmilax* also have numerous stamens. Sect. *Coilanthus* is closely related to *Heterosmilax* according to the previous studies mentioned by Koyama (1984), which is supported in this work, although the bootstrap value is not exceptionally high. In sect. *Macranthae*, most species form a clade, which implies somewhat close relationships between species although bootstrap values do not support this.

It is immature at the present time to subdivide naturally *Smilax* into sections based on morphology, though there are a few clades of species that can be defined by habit or vegetative features.

3.4   Geographic implications

Cameron & Fu (in press) have indicated clear differentiation between Old World and New World species in the *Smilax* on their ITS tree. However, our morphological tree shows a more complicated distribution pattern. It presents several mixed clades which nest species from different continents, but only two of them are supported by bootstrap values (as indicated by arrows in Fig. 1). One is the herbaceous *S. riparia-S. herbacea* clade with 93% bootstrap value, which is the evidence of migration from the Old World to the New World. Fu et al. (2005) have discussed the historical biogeography of this clade in detail. They attribute the disjunct distribution to Pleistocene vicariance events separating contiguous transcontinental populations following migration from East Asia to North America, as documented for a number of flowering plant groups (Boufford & Spongberg, 1983).

The other mixed clade with 62% bootstrap value contains *S. sieboldii*, *S. scobinicaulis*, *S. hispida*, *S. californica* and *S. bona-nox*. All these species (except *S. bona-nox*) together with a fossil taxon *S. lamarensis* Knowlton from Wyoming were presumed to have experienced migration from Asia to North America by Norton (1916). On the contrary, Cameron & Fu (in press) conclude according to their ITS analysis that the presence of *S. sieboldii* and *S. scobinicaulis* in Asia was a result of migration from North America. Our study also shows that the two Chinese species, *S. sieboldii* and *S. scobinicaulis*, were derived from North American species *S. hispida*, *S. californica* and *S. bona-nox* (see Fig. 1). Furthermore, *S. californica*, the most northwestern American species, has a basal position in this mixed clade, which favors Cameron and Fu's hypothesis based on ITS analysis (Cameron & Fu, in press).

This study gives an outline of phylogenetic relationships within the Smilacaceae, and thus further works on the Smilacaceae are badly needed. We are now generating more molecular data adding to the phylogenetic analysis.

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Smilacaceae   79   (   ),
Philesia Comm. ex Juss.   *Lapageria* Ruiz & Pav.   ,   47
                              ,   (NJ)   ,
                                        (1)*Ripogonum*   *Smilax* +
Heterosmilax   ,   (   )*Ripogonum*
; (2)   ,   sect. *Coilanthus*
                    (88%)   ,   ; (3)   6
                              ,   sect. *Nemexia*
(93%); (4)   ,   ,
        ,
                    ;   ;   ;   ;   ;   ;